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Study of the toxicity of ethanolic extract of *Schrankia leptocarpa* D.C in the treatment of dental caries in Côte d'Ivoire

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ABSTRACT

Schrankia leptocarpa (*S. leptocarpa*) is a plant found in the Mimosaceae family. It is used in the treatment of dental caries in Côte d'Ivoire. Because of the direct contact of the decoction with the cells of the mouth and the risk of swallowing it, we have been interested in the study of the toxicity of *S. leptocarpa*. The aim of our study was to assess the risks linked to the toxicity of plant during the traditional treatments. To achieve this goal, cytotoxicity and acute oral toxicity tests of the ethanolic extract of *S. leptocarpa* (P) were carried out at different concentrations on Vero E_6 cells from the black monkey kidneys of West Africa and on Swiss mice. This study showed a cytotoxic effect of extract P on Vero E_6 cells for all the concentrations ranged from 10 to 2.5 µg/mL from the second day. This extract has been found to be no-toxic with an LD_{50} greater than 5000 mg/kg. At the end of this study, it was shown that *S. leptocarpa* could be used in the treatment of dental caries without any fear of toxicity.

Keywords: Cytotoxicity, acute toxicity, Vero E_6, Swiss mice, LD50.

INTRODUCTION

For socio-cultural and economic reasons, a large part of the African population uses medicinal plants (OMS, 2001). The therapeutic use, accidental ingestion or the confusion of these plants with other edibles, are the main causes of frequent accidents in the world (Fourasté, 2000). Thankfully, the use of medicinal plants has been especial and widely spreaded in the treatment of dental caries (Bayeli et al., 2019). Tooth decay is a microbial condition that is manifested as a progressive

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destruction of the hard tooth tissue (Vidal, 2019). It is ranked as the third plague in the world's (Galmiche, 2011), and affects more than 60 to 90% of students and almost 100% of adults (OMS, 2012). After the results of ethnobotanical surveys carried out in the regions of Mé and Bélier, located respectively in the south and in the center of the Ivory Coast, our choice fell on Schrankia leptocarpa DC., A Leguminosae-Mimosoideae for this study. This plant is traditionally used as a mouthwash in the treatment of tooth decay (Atsain, 2017). The antibacterial tests of the aqueous and hydroethanolic extracts of *S. leptocarpa* carried out showed that the ethanolic extract P had better activity with respect to the bacterial strains of *Streptococcus mutans*. In addition, this extract has shown very good analgesic activity (Atsain, 2017). To recommend the traditional use of *S. leptocarpa*, cytotoxicity and acute oral toxicity were evaluated.

MATERIALS AND METHODS

Plant material

For our study, the whole plant of *S. leptocarpa* was used as the plant material. It was harvested in 2014 in the south of Côte d'Ivoire in the Mé region, more precisely in Abié and identified at the National Floristic Center (CNF) in Abidjan (Côte d'Ivoire) under the number UCJ009858. The plant material was then dried under permanent air conditioning (18 °C) for a week, then reduced into a fine powder using a grinder (Laboratory Blender®) at the Laboratory of Bio-Organic Chemistry and Natural Substances of Nangui Abrogoua University. The powder was then sampled for the different analyzes.

Animal model used in the experiment

The experimental animal model was extracted from Vero E_6 cells; originally extracted from the kidney of the black monkey; this model was used to evaluate the cytotoxicity of our extracts. Moreover, the female Swiss albino mice aged about 4 to 6 weeks, healthy, of weights between 19 and 27 g, from the Pro 5 animal facility at Félix Houphouët-Boigny University in Cocody (Abidjan / Côte d'Ivoire), were used to carry out the test for the acute oral toxicity of the extract. The animals were treated in accordance with the recommendations of bioethics (OCDE, 2001a).

Extract preparation

The extraction was carried out according to the method used by Guessennd-Kouadio et al. (2013). 200 g of the powder were introduced into an Erlenmeyer flask topped with a condenser, 1 L of ethanol (96%) was added to the flask and the whole was brought to a reflux boiling for 30 min on a sand bath. After cooling, the decocted was filtered. The filtrate was concentrated at 60 °C under vacuum using a rotary evaporator and dried out in an oven for 3 days at 50 °C to obtain the total ethanolic extract P, which under tight protection was kept in the refrigerator at 4 °C.

Study of cytotoxic activity

Cell viability test

For this test, a slight modification of the Musa et al. (2014) method was used. For this test, a concentration of 0.41 mg/mL was prepared for the extract in 10% MEM medium (Minimum Essential Environment). Vero E6 cells grown in 75 cm^2 culture dishes were individualized from trypsin-ethylenediaminetetraacetic (EDTA) (0.025% trypsin, 0.01% EDTA) (25300-054). A number of $0.5x10^5$ cells/mL was cultured in 2 mL of MEM growth at 10% in 11 culture tubes of 2 cm^2 and incubated for 48 h at 37 °C, under 5% of CO_2. Before the test, a tube was trypsinized in order to know the exact number of cells ($0.63x10^6$ cells/mL in the different tubes). 2 mL of growth medium (10% Fetal Calf Serum: FCS) and 50 µL of the extract were added to the tubes containing the cell mats, except for the controls, then returned to the oven at 37 °C, under 5% of CO_2. Which gives us an extract concentration equal to 10 µg/mL. A daily reading of an experimental tube and a control

tube using an inverted microscope was taken every morning for 5 days.

Cell sensitivity test

The method used is a slight modification of that carried out by Ambe et al. in 2016. A range of extract concentrations from 20 µg/mL to 0.039 µg/mL were prepared using the double dilution method. The Vero E_6 cell carpet contained in a 75 cm^2 culture dish was trypsinized and a suspension of 3.6×10^6 cells/mL was produced. The Vero E6 cell layer contained in a 75 cm^2 culture dish was trypsinized and a suspension of 3.6 × 106 cells/mL was produced. The different concentrations were distributed in the wells of a microplate wells of a 96-well microplate, at a rate of 100 µL of an extract concentration per well. Then a quantity of 100 µL of the cell suspension was in the wells. The control consists of cell suspension only. A number of 10 test wells and a control well constituted one test. This operation was repeated 3 times on the plate. A total of 5 plates were used. The different microplates were incubated at 37 °C in a 5% CO_2 atmosphere. The daily reading of a plate was carried out for 5 days using an inverted microscope in order to observe the effect of the extract on the cells. At the end of the readings, the reaction medium and the microplates were rinsed with a phosphate-buffered saline (PBS) solution. 100 µL of violet crystal dye were placed in the microplates and left for 24 hours on the bench. Then, the dye was aspirated and the microplates were rinsed with distilled water. The presence of plaque and the extent of their surface area, reflected by the absence of blue coloration in the wells, demonstrates the cytotoxic effect of the different concentrations of the extracts.

Assessment of the acute toxicity of *S. leptocarpa*

Experimental protocol

For the study of toxicity, the method 423 according to OECD (OCDE, 2001b) was carried out. At each stage of the test, 3 mice were used. These mice were females and were fed only with water for 3 to 4 hours before the test. As we did not have any information on the extracts to be tested, an initial concentration of 300 mg/kg of BW was administered to the animals by oral route an initial concentration of 300 mg/kg of BW was administered to the animals orally at a rate of 100 mL per 100 g of BW. None observed no deaths, the dose of 2000 mg/kg of body mass was administered. Two hours after the administration of the extracts, the mice were observed 30 min, 4 h, 24 h and daily for 14 days. Signs of toxicity observed in animals have been registered.

Statistical analysis of the results

The comparison of the results between the control and the tests was made using the ANOVA variance, followed by the Tukey test. The kruskal Wallis, Freidman tests and excel logicial were also used. The results obtained were expressed as an average ± standard deviation. $P<0.05$ was considered statistically significant, and $P<0.01$, very significant.

RESULTS

Yield of the extractions

For 200 g of organs removed, the ethanolic decoction of the whole plant has an extraction yield equal to 10.77%. This extract has a low yield compared to that of *T. glaucescens* (14.8 g) obtained from 100 g of organ (Konan et al., 2016).

Cytotoxicity studies

Measurement of cytotoxicity

The percentages of viability of Vero E_6 cells after the treatment for 24 to 120 h with 10 µg/ml of extract are presented in Figure 1. We observe that after 24 hours of presence of ethaolic extract P in the culture medium, no influence on the viability of the cells was observed. On the other hand, from the 2nd day to the 5th day, P causes a decrease in the viability percentage of between 0 and 20.

Figure 2 shows the shape of cells during the experimentation. The observed granule

shape in (Figure 2 B) is caused by toxic effect of P extract. Figure 2 A shows Véro E_6 normals cells shape.

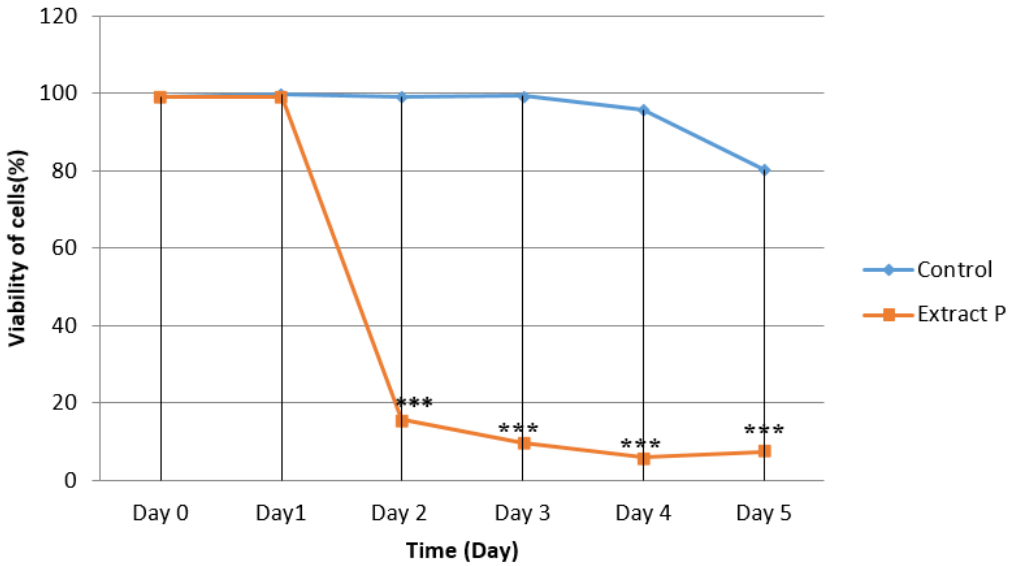
Sensitivity of cells

The sensitivity test was performed following a modification of that used by Irie-N'guessan et al. in 2011. This sensitivity test, based on the presence or absence of blue coloration in the wells of the experimental microplates, confirms the tests previously carried out. It was observed an increase in the toxicity of the extract over time and in a straight correlation with the concentrations used. Extract P showed a cytotoxic effect up to 2.5 µg/mL (Table 1).

Acute oral toxicity

Toxic effects

The behavior of all the experimental mice after oral gavage with the crude extract at 300 and 2000 mg/kg of body weight (BW) for 30 min is recorded in Table 2. After 4 h, 1 day until the 14th day, no signs and deaths were observed (Table 2).



$p < 0.001$; n=0.63x106 cells/mL

Figure 1: Viability of Vero E_6 cells.

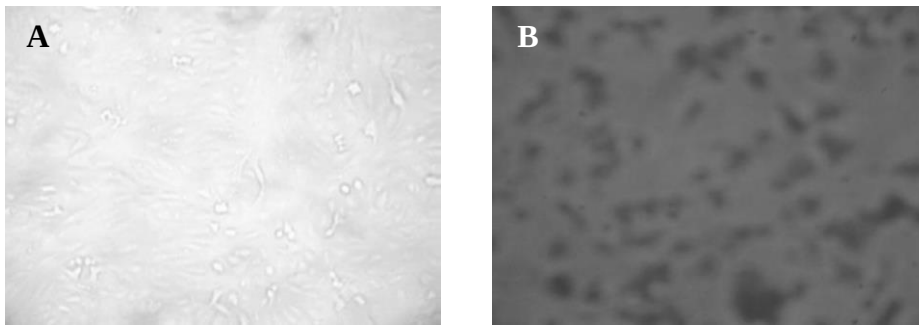


Figure 2: Véro E6 Cells before (A) and after experimentation (B).

Table 1: Sensitivity of Vero E_6 cells to the extracts as a function of time.

		Concentration of extracts (µg / mL)										
Days and substances		10	5	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.0195	0
D1	**P**	±	±	-	-	-	-	-	-	-	-	-
D2	**P**	+	+	+	-	-	-	-	-	-	-	-
D3	**P**	+	+	+	-	-	-	-	-	-	-	-
D4	**P**	+	+	+	-	-	-	-	-	-	-	-
D5	**P**	+	+	+	-	-	-	-	-	-	-	-

±: Appearance of cytotoxic effect; +: presence of cytotoxic effect; -: Absence of toxic effect.

Table 2: Signs of toxicity.

Extract	Concentration (mg/kg B.M.)	Observations
P	300	Tremor, sleep, rapid heartbeat for some time, restlessness
	2000	Tremor, sleep, increased heart rate for some time, restlessness, winding effect

P: ethanolic extract of the whole plant.

DISCUSSION

The P extract (10.77%) has a low yield compared to that of T. glaucescens (14.8 g) obtained from 100 g of organ (Konan et al., 2016).

For the cytotoxicity assessment, the decrease in the number of cells in the control tubes could be due to the lack of growth nutrients in the culture medium. The ethanolic extract tested showed a toxicity against the Vero E_6 cell line on the second day. Indeed, this led to the death of more than 70% of cells at 10 µg/mL. According to the work of Coulerie (2012), the extracts causing cell mortality greater than 30% at a concentration of 10 µg/mL are considered to be cytotoxic. Extract P, having a low viability percentage, highlights a good cytotoxic effect (Konan et al., 2016). The sensitivity test conforms to the results obtained during the cell viability test. In fact, the concentration of 10 µg/mL caused the lysis of the cells in the wells 24 hours later and was accentuated 48 hours later. In addition, the concentrations 5 µg/mL and 2.5 µg/mL were also the cells.

The signs of toxicity observed 30 minutes after the animals were stored disappeared 1 hour after the administration of the ethanolic extract P before the animals had access to food. Regarding the acute oral toxicity of the plant, no death of mice was obtained. For this, the LD50 value would be greater than the quantity administered (Gome et al., 2011). According to the OECD classification in 2001b, LD50 would be greater than 5000 mg/kg of BW, deducing that the ethanolic extract would belong to category 5 of toxicity.

Conclusion

The ethanolic extract P has cytotoxic potential at a concentration of 2.5 µg/mL to 10 µg/mL on Vero E_6 cells. It also causes signs of toxicity at 300 and 2000 mg/Kg of BW. The LD50 being greater than 5000 mg/Kg of BW, the extract could be recommended as a

mouthwash at a concentration of less than 2.5 µg/mL.

COMPETING INTERESTS

The authors declare they have no competing interests.

AUTHORS' CONTRIBUTIONS

The authors have all contributed in one way or another to the making of this work. Thus MRA-A, ABK, JAM-B and Y-AB carried out the chemical tests. Cytotoxicity was performed by MRA-A and EVA. As for acute toxicity, it was carried out by MRA-A and EK. Improvement and final manuscript approval was carried out by ANK-G, JAM-B and Y-AB.

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